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BY

ROBERT CHAMBERS, M.A., PH.D.

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ERRATA.

Page 16, line 16, for "Fig. 13" read "Figs. 13 and 20".

Page 28, third line from last, for "chromosome" read "chromatin".

Page 30, sixth line from last, for "Jen. Zeitschr., 37" read "Jen.
Zeitschr., N. F., 30".

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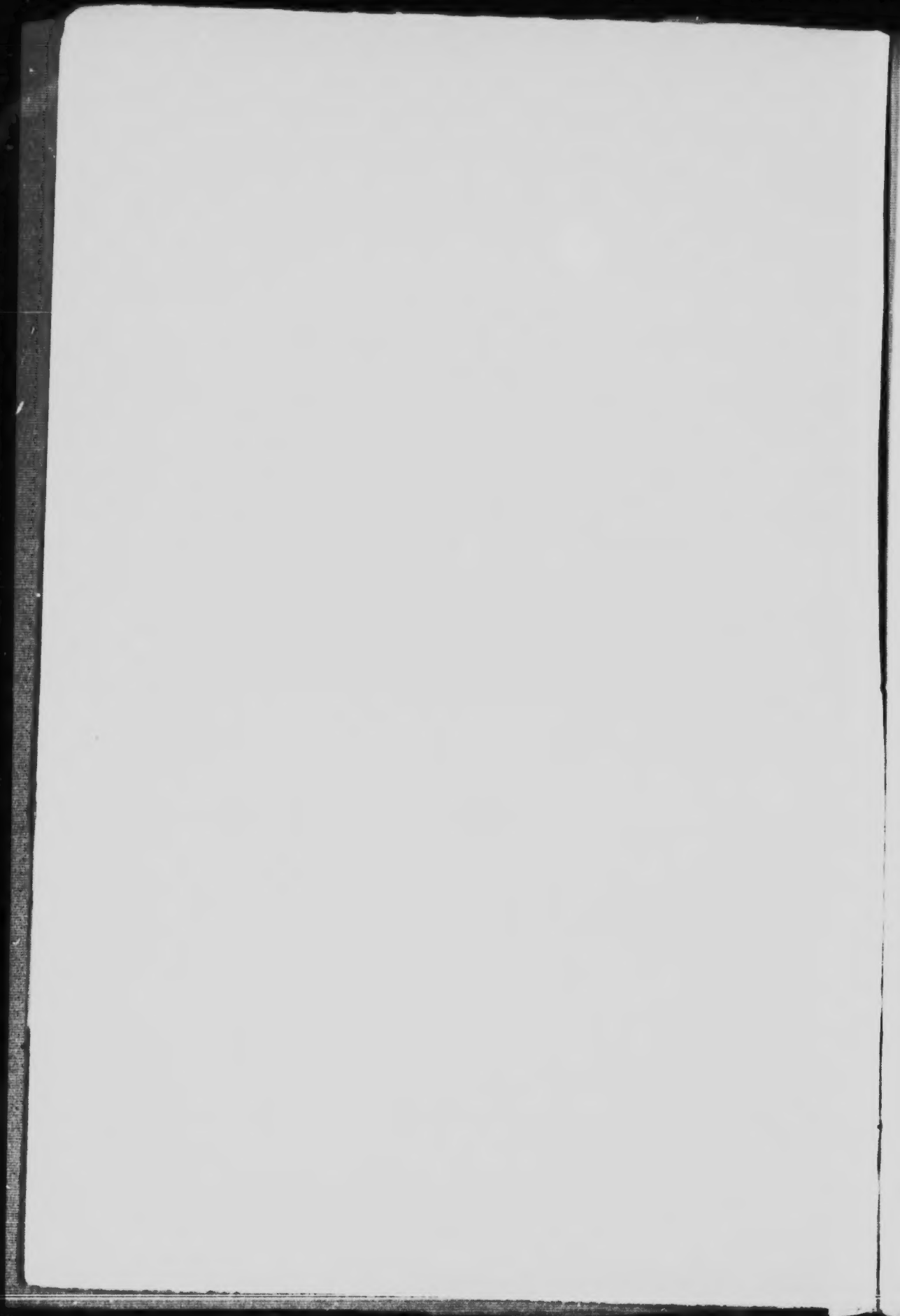
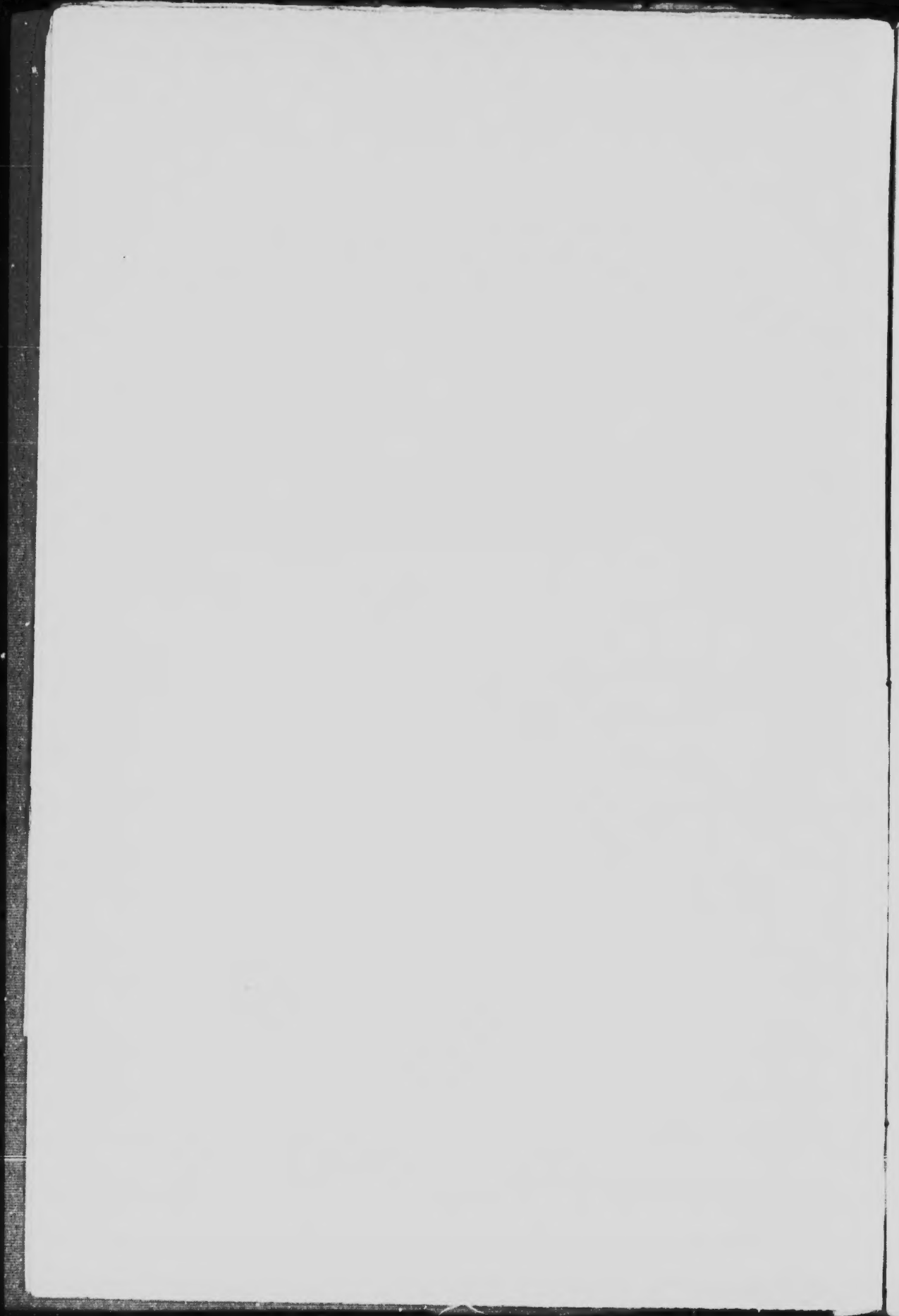


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EGG MATURATION, CHROMOSOMES AND SPERMATOGENESIS IN CYCLOPS.*

I.—INTRODUCTION.

Most species of Cyclops show some degree of periodicity in their breeding habits. *Cyclops viridis* and its close relatives, *C. parvus*, *brevispinosus*, and *americanus*, with which this paper has most to do, may be found in sexual activity all the year round. They are, however, especially active during the spring months.

During copulation, the male, which is about one-third to one-half the size of the female, attaches a pair of spermatophores to the median ventral aperture of the seminal receptacle which lies in the first abdominal segment of the female (cf. Wolf '05). The peculiar ejaculatory bodies in the spermatophores then swell and drive the spermatozoa into the seminal receptacle.

Oogonial mitoses occur periodically so that there is always a number of cells in the same stage being gradually carried onward through the ovary. When the oocytes pass into the paired oviducts they grow very rapidly and, by distending the several branches of the oviducts, cause them to occupy the greater part of the interior of the cephalothorax. A gelatinous material fills the distal ends of the two oviducts, and as the eggs pass out this is pushed out ahead to form a large distensible sac in which the eggs come to lie (cf. Gruber '78). The eggs are fertilized as they roll in rapid succession out of the oviducts. Development is, therefore, comparatively uniform for all the eggs in both egg sacs.

A female may lay six to seven batches of eggs, all of which may be fertilized by the spermatozoa derived from one male. These batches, consisting of thirty to fifty eggs, are

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generally deposited once every two to four days. The eggs are laid usually in the early hours of the morning (cf. Haecker '97). Ova showing maturation divisions I secured between 2 and 5 a.m., after having isolated the night before a number of gravid females. These are easily distinguishable because the yolk-laden eggs in their interior makes them appear black in transmitted light.

Spermatogonial mitoses exhibit a certain degree of periodicity which is by no means so marked as in the oogonial divisions.

Males are usually found in abundance together with the females. Their span of life, however, seems to be shorter than that of the females, and periods occur when few or no males are to be found.

II.—MATERIAL AND METHOD.

An easy method for collecting an abundance of material is the following: Scour the ditch or pond with a fine cheese-cloth net; invert the net in a jar of water and shake so as to release the catch into the water; pour the water through a coarse sieve so as to get rid of leaves and larger undesired objects. Then pour the water into a funnel plugged with absorbent cotton. When the water has drained through, pick out the plug of cotton with a pair of forceps, turn it over and dip rapidly into the fixing-fluid. Most if not all the Cyclops will at once liberate themselves from the cotton and soon fall to the bottom of the fluid.

As fixing-fluids I have used sublimate alcohol in various proportions both hot and cold, picro-acetic alcohol (McMurrich), picro formol (Bouin), Zenkerformol, Gilson's mercuronitric mixture, vom Rath's picro-aceto-osmic, Flemming's strong solution, Meves' modification of Flemming, and Carnoy's fluid (improved formula: glacial acetic, 1; absolute alcohol, 1; chloroform, 1; the mixture saturated with corrosive sublimate).

For the early stages in the ovary and for spermatogenesis, I found nothing so good as Flemming's strong solution. For oviduct eggs (showing late diakinesis figures) warm sublimate

alcohol (100 c.c. 70% alc., 5 grms. corros. subl., 0.2 grms. NaCl.; cf. Braun, '09), picroformol, and Carnoy's improved fluid gave very good figures. For ova in the egg sacs, showing maturation and early segmentation stages, I found Carnoy's improved fluid to be the best.

Material in warm (40°C.) sublimate alcohol was left for about 20 to 30 minutes, then removed to 70% iodized alcohol. In Carnoy the objects were allowed to remain about 3 to 4 minutes when they sank. They were then at once washed in 70% iodized alcohol. Sections were made from 3 to 10 μ in thickness. The nucleus of an ovum of *C. parvus* in the late diakinesis stage is, on the average, about 15 μ in diameter.

As staining reagents, Heidenhain's iron haematoxylin was found to be the best for the oogonial stages and for spermatogenesis. Iron haematoxylin, Delafield's haematoxylin, and Babe's safranin followed by light green were all used to advantage for staining late oocytes and the maturation and segmentation stages.

Investigation for this paper was begun in the University of Toronto, carried on at Woods Hole and completed in the Biological Laboratory of Columbia University. I wish to acknowledge my indebtedness to Professor F. R. Lillie for his kind courtesy in giving me a table at Woods Hole during the summer of 1911, and to Professor E. B. Wilson and Professor T. H. Morgan for the privilege of working in the Columbia Biological Laboratory and for their highly esteemed counsel.

III.—THE CYCLOPS "TETRAD".

Four principal methods have been described to explain the formation of tetrads preparatory to the maturation divisions. These I have attempted to show in Text-figure 1.

Text-fig. 1, Row 1.—The parasynaptic pachytene filament (*a*) splits to form two parallel filaments (*b*) presumably the same filaments that went into parasynapsis, except that during synapsis they may have interchanged material. Each filament then splits again longitudinally but in a plane

	SYNAPSIS		Metaphase		Tetrad	Anacaryosis
	a (Pachytene)	b (Diplotene)	Early	4		
1.						Grégoire, '04, '07; Strasburger, '09; Schreiner, '04, '06; Wisniewski, '07; Sainmont, '08; Janssens, '01.
2.						Montgomery, '03; Farmer and Moore, '04, '05; Jung, '05.
3.						Godschmidt, (Dicrocoelium), '04; Dublin, (Pedicellina), '05; Ishikawa, (Diapomus), '03; Rüchert, (Cyclops), '04.
4.						Braue and Matzsch, (Copepod), '09, '10; Tretjakoff, (Acania), '04; Jörgensen, (Sycon), '00.

Test-figure 1. Different interpretations for the formation of tetrads: 1, through parasyngapsis; 2, 3, and 4, through telosynapsis. In 1, 2, and 3 one of the maturation divisions is reductional. In 4 both maturation divisions are equational, the cross-entire taking no part.

at right angles to that of the first split (*d*). This tetrad may take on a variety of shapes according to the greater or less divergence along one or the other of the splits and also according to the varying thickening and shortening of the four rods, forming rings, crosses, etc. (*e*).

Row 2.—The telosynaptic pachytene filament (*a*) splits lengthwise to form two parallel filaments (*b*). These fold on themselves (*c*), along the line of union of the original telosynaptic filaments. The filaments then break at the bend to produce a tetrad (*d*) superficially resembling that of (1) and exhibiting the same variety of shapes (*e*).

Row 3.—The telosynaptic pachytene filament (*a*) splits as in (2). No folding, however, occurs. The filaments break at the point of synaptic union to form a simple tetrad (*d*). One maturation division is along the longitudinal split, the other along the cross-suture.

Row 4.—The telosynaptic pachytene filament (*a*) splits twice longitudinally. The resulting filaments then break along the line of synaptic union to produce a *ditetrad*. The transverse break, or cross-suture (*Querkerbe*), takes no part in the maturation divisions. It is interpreted by Haecker, Matschek, ('10), and Jörgensen ('10) as the point of union, in pairs, of somatic chromosomes in the early germ-tract cells. They thus assume that the chromosomes throughout the germ-tract exist in the haploid number.

For *Cyclops strenuus*, Rückert ('94) described the synapsis spireme as consisting of a single series of chromosomes joined end to end. After splitting longitudinally the spireme breaks up into rods half the somatic chromosomes in number. These longitudinally split rods (Text-fig. 1, row 3) exhibit a cross-suture which passes through their middle and indicates their bivalent nature. The first maturation division divides these tetrads along their longitudinal split and is equatorial, the second passes through the cross-suture and is reductional.

Haecker ('95) gave the same interpretation to the tetrads in *Canthocamptus*, a genus closely related to *Cyclops*.

For *Cyclops brevicornis*, however, he ('95b, '02) gave a different interpretation. This species, according to him,

Early.	Late.	1st Division.	Interkinesis.	2nd Division.
I.	II.	III.	IV.	V.
VI.				

Text-figure 2.—Haecker's interpretation of the method of synapsis and maturation in *Cyclops brevicornis*.

I. A bivalent chromosome $a b$ pairs with another $a b$. II. Each splits lengthwise. III. A cross-break (*Overbridge*) appears, rendering apparent the bivalency of each chromosome. IV. In the first maturation division the longitudinal halves of each chromosome separate. V. During interkinesis the chromosomes fuse in pairs so as to form cross-like structures. VI. The second maturation division passes through the original cross-suture so that each definite chromosome in the mature egg is both grandmaternal and grand-maternal in origin (syngamixis).

possesses twenty-four somatic chromosomes. In the germ-tract the chromosomes are twelve in number, each consisting of two chromosomes joined end to end. In the oocyte these twelve bivalent chromosomes arrange themselves into pairs (Text-fig. 2, I). Each one of a pair then splits lengthwise (II) and also breaks across the line of the original telosynaptic union (III). Each pair, therefore, is formed into a tetrad or two tetrads lying parallel to one another. During the metaphase of the first maturation division these tetrads are so arranged, six in each of two parallel planes, that every tetrad in one plane lies parallel to, and directly opposite, another tetrad in the other plane (III). He assumed that the six tetrads in the one plane are of paternal, and the other six of maternal, origin. In the first maturation spindle the longitudinal halves of each tetrad are carried to separate poles (IV), the division being homœotypic. During interkinesis, the two dyads of the formerly opposite pairs become contiguous and fuse at their middle points to form double V figures (V). The plane of division in the second maturation spindle, as in other copepods, passes through the cross-suture of the original tetrads, but because of the fusion of the dyads into double V figures, the chromosomes which go to the poles of the spindle are still bivalent, but consist of new sets of halves, e.g., *a n*, *b o*, etc. (VI). A synmisis of both grandpaternal and grandmaternal chromatin elements is thus produced within the pronucleus.

This assumption of synmisis during interkinesis, based merely on the fact that the tetrads of *C. brevicornis* are peculiarly X-shaped, is elaborated by Haecker in order to account for the presence in the male or female pronucleus of both grandpaternal and grandmaternal chromatin elements. The assumption is made necessary because of Haecker's claim that gonometry obtains in the germ-tract nucleus,

Unfortunately for both Rückert and Haecker, recent investigations have placed the Cyclops tetrad in a very different light.

Braun ('09) in a study of some sixteen Cyclops species described paired chromosome rods which are longitudinally split and cross-sutured (Text-fig. 1, row 4). He also showed

that the peculiarly X-shaped tetrads of *C. brevicornis* are not produced during interkinesis but exist already in the oviduct egg and are due to the divergence at their ends of the longitudinal halves of the tetrads. Compare this with my own observation, Pl. 1, Fig. 4. The first maturation division separates the two parallel sets of tetrads; the second separates the diverging longitudinal halves of the tetrads.

Matschek ('10) made an extensive study of tetrads in species belonging to six families of the Copepoda. He agrees with Haecker and Rückert in regard to the origin of the tetrads from an incompletely segmented longitudinal spireme. He confirmed Braun's discovery, however, that Rückert's tetrads are really ditetrads or octads (Text-fig. 1, row 4). The oogonial chromosomes being twelve in number, there are six ditetrads in the oviduct egg. The primary longitudinal split is much broader than the secondary, so that the ditetrad gives the appearance of two tetrads lying parallel to each other. The two maturation divisions divide the ditetrad along the two longitudinal splits. The cross-suture being interpreted as the place of conjunction of two chromosomes and taking no part in the maturation divisions, Braun and Matschek consider both maturation divisions equational.

Lerat ('05) discountenanced the existence of the cross-suture in Cyclops. He worked on a variety of *Cyclops strenuus* found in ditches, the same species studied by Rückert. According to Lerat's description leptotene filaments in the young oocyte conjugate in pairs by parasynapsis to form pachytene filaments. These subsequently split along the line of conjugation (cf. the split spireme of Haecker, Rückert and Matschek). As the filaments shorten and thicken, they form paired chromosomes. These show no signs whatever of a cross-suture. Lerat did not go beyond the metaphase of the first maturation division.

It is interesting to note here that Braun mentions the case of a winter form of *C. strenuus*, living in ponds which dry up in the summer. In this form the chromosomes are long and U-shaped and the cross-suture is barely noticeable. Other individuals of the same species, living in lakes or in small ditches throughout the summer, possess chromosomes

with a very distinct cross-suture. Lerat may have studied only the winter form. The apparent contradiction in the statements of Lerat and Braun concerning the cross-suture may also be due to the fact that the two used different killing and staining fluids. Lerat used Gilson's mixture and Heidenhain's iron haematoxylin. Braun used sublimate alcohol and Delafield's haematoxylin.

Miss Krimmel ('10) is the latest to describe tetrads in Copepoda. She made a study of the generative cells during the late embryonic development of *Diaptomus coerul-eus*. In her preparations she finds cross-sutured chromosomes both in oogonial and in somatic cells. The chromosome number in the germ-tract she claims to be thirty-two. In the somatic cells, however, she finds the number to vary anywhere from sixteen to thirty-two. Her paper is a preliminary report. We may defer criticism therefore, until her complete paper is published.

Tetrads whose cross-suture takes no part in either of the two maturation divisions have been described in few forms outside the Copepoda.

Tretjakoff ('04a) in the egg maturation of *Ascaris megalocephala bivalens* described two chromosome groups each consisting of two parallel chromosomes, each of which is longitudinally split. A transverse suture often appears in the middle of these split chromosomes which later on disappears without taking part in the maturation divisions.

In a later paper ('04b) Tretjakoff described the shape of the prophasic spermatocyte chromosomes of *Ascaris* that may well account also for the transverse suture he saw in the prophasic egg chromosomes. The chromosomes first appear as ribbon-like bodies with thickened ends. Later their middle region becomes so narrow as to consist of a mere thread connecting the two ends. Tretjakoff suggested that the middle region of the chromosomes consists of trophochromatin, and the two ends of idiochromatin. During the maturation stages the trophochromatin disappears, thus giving the chromosomes the appearance of being broken in the middle.

Boveri ('04) and Montgomery ('04), however, and more recently Griggs ('06), mention no such suture in the *Ascaris*

forms studied by them. This and the general appearance of Tretjakoff's figures make one rather sceptical of its normal occurrence in *Ascaris*.

Marcus ('06) in *Ascaris canis* described a cross-suture giving the tetrads the appearance of ditetrads. Edwards ('11), however, in a closely related species, *Ascaris felis*, found nothing of the sort.

A very recent paper which describes a condition similar to that of the Cyclops tetrad as explained by Haecker is one by Blanckertz ('11). Blanckertz describes eight "chromosomes" in the first maturation prophase of *Ascaris megalocephala univalens*. The eight fuse end to end, in pairs, to form the four elements of the *Ascaris* tetrad. Each element of the tetrad is thus bivalent in the sense of Haecker's bivalent Copepod chromosomes.

In Sponges, Jörgensen ('09) describes in a *Sycon* eight tetrads which appear in the first spermatocyte equatorial plate. During metaphase I the tetrads split into dyads which in the anaphase appear again as tetrads. In metaphase II these divide again into dyads. The spermatid chromosomes thus appear to be bivalent.

Tetrads have been described by Buchner ('09) as being found also in the oogonial cells of *Gryllus*.

The artificial production of such structures in somatic cells and in the egg (Haecker, '00; Schiller, '08; Della Valle, '09) through the action of the strychnine and other poisons should render us cautious in accepting statements as to their normal occurrence in cells, at least where chromosomes are known to be diploid in number.

My own observations have been limited to *Cyclops americanus*, *C. parvus*, and *C. brevispinosus*.

Double chromatin filaments in the reduced chromosome number come out of the synizesis stage and persist as such throughout the growth period of the ovum. The two elements of the double filament, which are at first close together, separate more and more as they contract to form short paired rods. During the late prophase of the first maturation division these paired rods are scattered throughout the nucleus (Pl. I, Fig. 4.)

When the nuclear wall breaks down, the paired rods are drawn into the biserial arrangement (Figs. 5-10).

From a careful study of a large number of sections of this stage in *C. americanus*, *parvus* and *brevispinosus*, I am convinced that the so-called *Querkerbe*, or cross-suture, is not an actual break but rather a clear area of the chromosome due to the faint staining power of that region. The chromosome rod is somewhat larger at its two ends than along its middle. If we assume that this narrower, more faintly staining region is easily broken through when effected by killing reagents, we may account for the presence of a cross-suture in so many recorded instances.

Fig. 9 gives the side view of several chromosome pairs from different oviduct eggs in the same individual. All were found on the same slide so as to insure as far as possible uniformity in fixation and staining action.

That the cross-suture, or rather the clear area, frequently does not lie in the middle of the chromosome rod has been commented upon by Rückert himself ('94, p. 308). An instance of this is to be seen in Fig. 9a. It is noteworthy that the clear area is always in the same region for the two rods of the same pair. Fig. 9b shows one rod completely broken into two portions, probably an artifact, for its mate is intact. Fig. 9c shows the clear area in the middle. Fig. 9d exhibits a condition very frequent in my preparations when the rods show no sign whatever of a clear area.

During all stages except that of the biserial arrangement the chromosomes are U-shaped. Here only do the two arms of the chromosome stand out to form a more or less rigid rod. May not the same force that holds them in this way cause a massing of the chromatin substance toward the two ends? This would leave an achromatic substance in evidence at the middle.

The suggestion that a chromosome consists of two substances, an achromatic framework or substratum and a chromatic substance, is in accordance with the view of Bonnevie ('11) for *Allium* and *Amphiuma*, that a chromosome consists of an achromatic core around which is coiled a chromatic spiral thread.

IV.—MATURATION OF THE OVUM.

Just before the eggs pass out of the oviduct a second nuclear membrane differentiates, enclosing a much smaller area than the former nuclear membrane of the germinal vesicle (Pl. 1, Fig. 10).

Upon fertilization, as the eggs pass out of the oviduct, this secondary nucleus approaches the periphery of the egg. No typical metaphase figure is ever formed, the chromosome pairs in the biserial arrangement passing directly into anaphase I (Figs. 11, 12, 13).

As Matschek has already observed, the first polar body is formed within two to three minutes after the egg is laid. Very little, if any, cytoplasm is given off with the polar body. As the nucleus moves to the periphery of the yolk-laden egg, it leaves a path of cytoplasm behind it (Fig. 11). When it reaches the periphery it protrudes, pushing out in front of it a membrane (Fig. 13). During the late anaphase the chromosomes assume their original U-shape, and from now on no sign whatever of the clear area in their middle can be seen. The nuclear membrane remains intact until a constriction at its middle occurs which finally cuts off the polar body.

Matschek's figures are remarkable because of the peculiar distinctness with which the "cross-suture" is depicted during maturation divisions. By examining my objects with the powers used by Matschek (Zeiss Imm. 1/12, Comp. Oc. 12), I found that I could readily deceive myself as to the presence of a cross-suture by not taking into account the U-shape of the chromosome, for the middle portion of the chromosome is left out of focus at the time that the two ends are in view.

That side of the nuclear membrane from which the polar body is constricted off shows a break for some time (Pl. 2, Fig. 14). It is soon repaired, however, to form a closed nucleus in which the split chromosomes arrange themselves for the second division by turning 90° on their axes. The split halves now are drawn asunder by spindle fibres which are distinctly visible (in contrast to those of the first maturation division) (Figs. 17, 18, 19). The metaphase figures

are similar to those of the oogonial chromosomes, the spindle fibres being attached medially or subterminally.

Fig. 20 shows the second polar body about to be constricted off. The chromosomes are somewhat massed together in the telophase. The nuclear membrane is still intact.

In Fig. 21, the polar body has been given off and the female pronucleus has receded into the egg, surrounded by a very indistinct membrane. The chromosomes are losing their definiteness of outline and will soon form the reticulum of the female pronucleus.

Fig. 22 is a polar view in *C. parvus* of the male and female pronuclei lying in the first segmentation spindle. The three chromosomes of one of the pronuclei, presumably the female pronucleus, are already definitely formed and are beginning to show signs of splitting.

Haecker and his pupils, also Rückert, and Ishikawa, have already described the remarkable autonomy of the male and female pronuclei during the earlier segmentation processes. The autonomy goes so far that one may often observe two almost complete spindles side by side each with its proper chromosomes.

V.—CHROMOSOME NUMBER IN CYCLOPS.

(a)—CHROMOSOMES IN THE GERM-TRACT.

There is no doubt that the chromosomes in the germ-tract cells are unreduced in number. Krimmel ('10) has shown this to occur in *Diaptomus*. In *Cyclops americanus* I have been able to make out ten U-shaped chromosomes in several tissue cells. That the chromosomes occur in the same number in the oogonia and spermatogonia of the same form may be seen from Fig. 1 and Figs. 26, 28, 29.

The conclusions of Krimmel and myself are contrary to the statements of vom Rath ('95) who observed thirty-two elements in the mitoses of the alimentary canal cells of *Anomalocera patersonii*, a marine Copepod, and sixteen

elements in the mitoses of the oogonia; and Matschek ('10) who figures an oogonial anaphase in *Cyclops fuscus* showing seven chromosomes, whereas in the biserial arrangement seven pairs are to be found.

Figs. 2 and 3 show the six oogonial chromosomes in *C. parvus*. The chromosomes are usually U-shaped and apparently very plastic in nature. During the oogonial metaphase they split longitudinally and in the anaphase the slender halves shorten to form thick, semi-curved chromosomes of about half the length of the mother chromosomes. In none of my preparations is there any figure approaching that of a tetrad such as Krimmel depicts in the *Diaptomus* germ-tract cells.

(b)—CHROMOSOME COUNTS IN DIFFERENT SPECIES.

Braun ('09) and Matschek ('10) have ascertained the chromosome counts for sixteen of the European species of *Cyclops*. Braun made a study of the relation between the chromosome number and the external specific characters of the species. Taking the condition of the fifth rudimentary foot and the number of antennal segments as criteria, he found in general that those which show least signs of rudimentation possess the greatest number of chromosomes. He made the following conclusions: (1) that the highest developed forms (e.g. many marine Cyclopidae) possess the greatest number, and those which are most highly specialized possess the smallest number, of chromosomes; and (2) that closely related species possess equal or nearly equal chromosome counts and, therefore, that the chromosome number may be used in the determination of species relationship.

As far as I have been able to make out, the chromosome counts for American species fit well into Braun's phylogenetic scheme. On the other hand, his statement that closely related species possess equal or nearly equal chromosome counts is quite untenable, at least for our American forms. The following table gives the species with their diploid chromosome counts as I have found them:

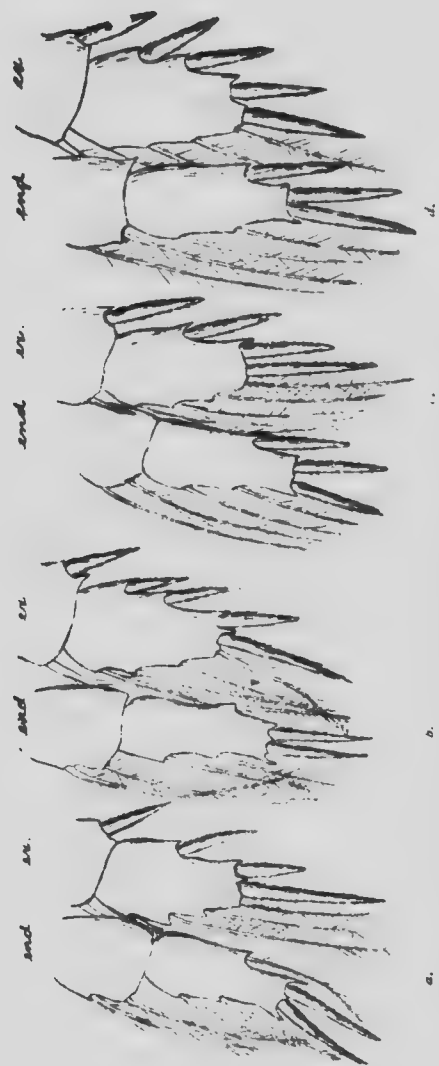
<i>Cyclops fuscus</i>	14
" <i>albidus</i>	14
" <i>bicuspidatus</i>	18
" <i>viridis</i>	12
" " var. <i>parcus</i>	6
" " " <i>americanus</i>	10
" " " <i>brevispinosus</i> ..	4
" <i>modestus</i>	8

C. fuscus, *albidus*, *bicuspidatus*, and *viridis* (cf. Chambers, '12) are morphologically identical with their European representatives. Their chromosome numbers are also identical with those found by Braun and Matschek. The other species mentioned in the table appear to have no European representatives. In the latest revision of the North American species of Cyclops, Marsh ('10) classifies *C. americanus*, *parcus*, and *brevispinosus* as American varieties of the European *C. viridis* Jurine. *C. viridis* (typ. sp.) has been described only by me as being found in American waters.

In their external features *C. americanus*, *parcus*, and *brevispinosus* are barely distinguishable, the only main difference being the number of spines on the terminal segments of the swimming feet (Text-fig. 3). It has been suggested (Byrnes, '10) that *parcus* and *americanus* are two phases in the life-history of the same form. I have discussed this matter elsewhere ('12). *Americanus* and *parcus* breed true for generations. Slight variations in the number of spines of the swimming feet among individuals of the same culture occasionally occur, but the chromosome number always remains constant.

Cyclops brevispinosus differs from *americanus* and *parcus* in frequently becoming sexually mature before the swimming feet attain the number of spines characteristic for that variety. We may therefore have a *parcus*-like form (Text-fig. 3, c) or an *americanus*-like form (Text-fig. 3, d) except for the presence of a spine on the outer side of the terminal segment of the endopodite of the fourth swimming foot and the presence in the cells of four chromosomes.*

* The caudal styles of *C. brevispinosus* are slightly thicker and shorter than those of *C. parcus* and *C. americanus*. All three are abundant in ditches and pools, although not associated in the same pool. The three appear equally infested with a unicellular green alga which often covers them completely.



Text-figure 3. - Terminal segments of the endopodite and exopodite of the 4th swimming feet in
 (a) *C. parvus* 2 spines on the endopodite; segment, 3 spines on the exopodite
 (b) *C. americanus* 2 " " " " 4 " "
 (c) *C. brevispinis* (*parvus* form) 3 " " " " 3 " "
 (d) *C. "* (*americanus* form) 3 " " " " 4 " "

C. viridis (typ. sp.), averaging 2.2 mm. in length, is the largest form in the *viridis* group (excluding occasional giant forms of all the varieties). It has twelve chromosomes. *C. brevispinosus* with four chromosomes comes next, averaging 1.6 mm. in length. *C. americanus* with ten chromosomes, and *C. parvus* with six chromosomes, come last and are barely distinguishable from each other in size.

The size of the chromosomes varies greatly in the three varieties, *C. brevispinosus* possessing by far the largest, and *C. americanus* the smallest, chromosomes. The proportions for the different forms are such that we could readily assume a relationship between the average sizes and the amount of their chromatin content.

An explanation of the discrepancy of chromosome number in closely related forms is offered by Wilson ('09) in the following words:—

"It seems to me a natural view that the nucleus consists of many different materials or substances that segregate in a particular pattern; that different chromosomes need not, however, represent a complete separation of different substances but are in many cases, perhaps in all, compound bodies; and that the particular form of segregation may readily change from species to species. Marked or even extreme changes might have taken place in the number and size relations of the chromosomes that would involve little or no change in the essential quality of the nuclear substance, and the apparent anomaly presented by differences in the chromosome groups of nearly related forms would disappear."

VI.—CHROMOSOME SIZE-RELATIONS AND ARRANGEMENT IN *Cyclops parvus*.

In *C. parvus* the six somatic chromosomes occur in three sizes, there being a pair for each size.

Pl. I, Fig. 2 shows the oogonial chromosomes on the point of being arranged in the equatorial plate. In spite of the fact that they are somewhat bent, one may readily pick out the pairs, two long, two medium-sized, and two short chromosomes. We may see here as Wilson ('06) has already pointed

out for *Anasa* and other Hemiptera that the chromosomes of a pair do not necessarily lie together in the nucleus, an assumption held by many botanists.

The constancy with which the chromosomes retain their relative size during the different stages of maturation may be seen from a comparison of Figs. 2, 6, 15, and 22.

During the biserial arrangement the chromosomes of a pair lie parallel to each other. An exception is the case of one of the pairs in an individual taken from a culture of *C. parvus*. This individual possessed an abnormal number of spines on the terminal segment of the external rami of the swimming feet, the number for the four feet being, 3, 4, 4, 3, respectively, instead of 2, 3, 3, 3, the characteristic number for *parvus*. The chromosomes of the oviduct eggs were found to be in the biserial arrangement, and the smallest pair showed a deviation constant for all the eggs, some fifty or eighty, in the oviducts. The two chromosome rods instead of lying parallel to each other, as was the case for the other pairs in this individual, lay almost at right angles to each other (Fig. 7). It is remarkable that this abnormal arrangement should be so constant for that individual.

VII.—SPERMATOGENESIS IN *Cyclops americanus*.

I.—LITERATURE.

Ishikawa ('93) described the spermatogenesis of a *Diaptomus* sp. He gives eight to be the somatic chromosome number. After the loosening of the synaptic clump he makes out, rather doubtfully, eight filaments. These shorten to form the definite chromosomes of the spermatocyte of the first order. He describes no pairing of the chromosomes. The first maturation division is equatorial and the second is reductional, four of the eight chromosomes going to one pole and four to the other. His evidence, however, is very doubtful.

Haecker has published no account of spermatogenesis in Copepoda except a brief mention in his paper of 1902. There he figures a longitudinal section of a young *Heterocope testis*

to illustrate his contention that the paternal and maternal elements of the nucleus in the grown cell keep more or less independent of each other. He figures numerous nuclei to show their dual nature as evidenced by their bilobed appearance and the possession of double nuclei. In two instances two independent spiremes are shown in one prophase nucleus. In the spermatid his figures show double nucleoli very prominently. In two of the spermatocyte nuclei he figures distinct tetrads.

Lerat ('05) gave an account of the spermatogenesis in *Cyclops strenuus*. Although he was unable to count the spermatogonial chromosomes, he assumed them to be unreduced in number. He claims that reduction takes place through parasynapsis as the chromatin filaments come out of the contraction stage. His studies went no farther than the anaphase of the first maturation division, but in that stage he figures the daughter chromosomes split lengthwise preparatory to the second maturation division. He found no sign whatever of tetrads.

In *Cyclops americanus* the testis is single and median, lying immediately under the dorsal wall of the thorax. From its anterior end two *vasa deferentia* rise, and after winding several times, one on each side of the alimentary canal, pass back to open, one on each side of the first abdominal segment.

2.—THE KEIMPOLSTER.

In old individuals a cup-shaped depression containing a disorganized mass is to be observed at the blind end of the testis (Fig. 24). This depression indicates the location of the Keimpolster, or primitive germ-cell group, from which the testis is derived.

In immature individuals the Keimpolster is a rapidly proliferating mass of cells (Fig. 23).

In young sexually mature individuals it appears as a syncytium containing a number of deeply chromatic nuclei rather irregularly disposed but chiefly arranged along the periphery. The nuclei are small, barely two-thirds the size of the largest spermatogonial nuclei. Heavy strands of chromatic material cause them to acquire a dense stain.

The absence of mitotic figures in the Keimpolster of all sexually mature individuals, and the fact that the Keimpolster is separated from the testis proper by a sharply defined boundary, renders likely the supposition that, after producing a number of spermatogonia, it becomes inert and soon disorganizes, the growth of the testis henceforth being due entirely to spermatogonial mitoses.

This Keimpolster corresponds to that described by Haecker in *Canthocomptus* and is, according to him ('95a), to Amma ('10), and to Krimmel ('10), the direct descendant of the germ-cells differentiated as early as in the first cleavage of the egg.

Lerat ('05) was unable to find a typical Keimpolster in *C. strenuus*. He describes an apical cell from which he assumed the spermatogonial cells were derived. It is much more probable that this "apical cell" is merely one of the spermatogonial cells and that he failed to find the true Keimpolster as it may have been already disorganized in the individuals studied by him.

3.—MULTIPLICATION ZONE.

The region following the Keimpolster consists of a large number of proliferating spermatogonia forming a mass of closely appressed cells. Lerat figures this region as a syncytium. My preparations, however, give clear evidence of definite cell boundaries (Fig. 24). The resting nucleus (Fig. 25) possesses an irregularly blotched chromatic reticulum. Division figures are periodically frequent (Fig. 27). Definite spindle fibres are plainly visible. The chromosomes in the equatorial plate are diploid in number and are more or less U-shaped (Figs. 28, 29).

The size of the cells varies greatly, owing partly to difference in time of growth and partly to the number of spermatogonial divisions that the cells have passed through, the nuclei and cells near the blind end of the testis (Figs. 25, 28) being considerably larger than those about to pass into the synzesis stage (Figs. 29, 30).

4.—SYNIZESIS AND SYNAPSIS ZONE.

The term *synapsis* is generally used indifferently by European writers for the massing of the chromatin filaments in a nucleus and their conjugation. The term *synizesis*, first proposed by McClung ('05), is much more applicable to the massing of the chromatin, while the term *synapsis* ought to be restricted to the conjugation of the filaments.

In *Cyclops* there is a decided *synizesis* stage. The chromosomes of the last spermatogonial division do not pass directly into the *synizetic* filaments, there being an appreciable zone of resting nuclei next to the *synizesis* region. Figs. 30 to 33 represent a number of contiguous nuclei in which one may see the gradation between the irregular network of the resting nucleus and the entangled mass of fine threads in the *synizesis* nucleus.

A distinct sub-spherical nucleolus is noticeable at this stage. It is always situated at one side of the *synizetic* mass. No bouquet-like orientation of loops can be distinguished but the threads are clearly leptotene filaments. The nucleolus never attains the great size and irregular shape seen in Lerat's figures. It is somewhat rounded in outline, rather small, and never shows the intimate connection with the chromatin filaments as figured by Lerat. Lerat's figures give one the impression of incomplete extraction of the haematoxylin stain.

The nuclei of the cells undergoing *synizesis* are never larger than the small last spermatogonial nuclei. Gates' interpretation ('09) that *synizesis* figures may be due to the growth of the nucleus unaccompanied by growth of the chromatin content, cannot apply, therefore, to the case of *Cyclops*.

No positive result was reached as to the likelihood of para- or telo-synapsis taking place during this stage. There seems to be no doubt, however, that *synizetic* nuclei containing leptotene filaments exist together with *synizetic* nuclei containing pachytene filaments. The two types of filaments are easily distinguishable, there being no intergradations such as Matschek claims to be the case in the

oogenesis of *Cyclops*. Nuclei with pachytene filaments (Fig. 34) are most numerous in the region farthest from the spermatogonial zone.

5.—EARLY AND LATE DIAKINESIS.

As the synizetic coil begins to loosen, the pachytene filaments give the appearance of being lumpy along their lengths (Fig. 34). Numerous short splits longitudinally arranged on the filaments soon appear (Fig. 35). The coil finally resolves itself into five long filaments each consisting of two filaments tightly twisted about each other (Fig. 36). The spirals untwist as these filaments thicken (Figs. 37, 38, 39) until the five paired definite chromosomes of the spermatocyte of the first order are formed (Fig. 40).

There is a slight growth of the cells during the synizesis and early diakinesis stages. In the oocyte there appears to be some connection between cell growth, which is enormous, and the simultaneous increase in size of the nucleolus which is very great. In the spermatocyte, on the other hand, where growth is comparatively slight, no appreciable increase in size of the nucleolus is to be observed.

The two elements of the bivalent chromosomes are distinctly elongate dumb-bell-shaped. In one individual I found several spermatocytes of the first order which contained four bivalent chromosomes and two single elements lying at some distance from one another (Fig. 41). Undoubtedly the two single elements are halves of the fifth bivalent chromosome which have accidentally broken apart.

6.—MATURATION.

The spindle in Division I is an ordinary one with conical poles and numerous fibres (Fig. 42) and with no resemblance to that in the maturation of the ovum. Insertion of the fibres is either subterminal or median. In metaphase the two halves of the bivalent chromosome usually break away first at one end (Figs. 42, 43). The split in the chromosomes for Division II appears during Anaphase I (Fig. 44). In telophase (Fig. 45) the chromosomes become somewhat massed

together but their distinctness is never lost during interkinesis. During this time the split in each chromosome becomes very prominent (Fig. 46), each half appearing distinctly dumb-bell-shaped. In Division II the chromosome halves are drawn away from each other much as in Division I (Figs. 47 and 48). The spermatocytes of the second order (Fig. 49) contain five slender dumb-bell-shaped chromosomes.

7.—SPERMIOGENESIS.

The more or less rigid dumb-bell-shaped chromosomes become U-shaped (Fig. 50). They then lose their distinctness of outline through the appearance of irregular projections over their surface (Fig. 51). These projections grow and develop in such a way that a hollow sphere of a reticular chromatin mass is formed (Fig. 52), similar to that described by Montgomery ('12) in the spermiogenesis of the *Peripatus*. The sphere is then drawn out into the form of a spindle (Figs. 53, 54). As the spindle lengthens, it becomes compressed from side to side. At the same time it increases somewhat in size, and the small amount of cytoplasm originally about the sphere appears to be sloughed off.

The spermatozoon in the *vas deferens* (Fig. 55) is a slender, faintly staining, finely reticular mass with a slight spiral curve and long tapering ends. In cross section it appears narrow ovate (Fig. 55a). In the seminal receptacle of the female the spermatozoa are often curled in the form of a corkscrew.

VIII.—SUMMARY.

EGG MATURATION IN *Cyclops americanus*, *parvus* and *brevispinosus*.

1. The oogonial and spermatogonial chromosomes are diploid in number.
2. The tendency for the chromosomes, both of the oocyte and of the spermatocyte, to assume a characteristic U-shape seems to be subordinated during the prophase of the first maturation division to a force which causes them to assume a more or less rigid rod-shape, somewhat swollen

at the ends. In the oocyte this massing of chromatin at the ends leaves a clear area in the middle of the chromosomes. Such a clear area does not appear in the spermatocyte chromosomes.

3. Both egg-maturation spindles are entirely within a nuclear membrane. The spindle fibres, attached subterminally or medially to the chromosomes, appear most distinctly in the second maturation spindle. The spindle poles are very broad so that the fibres appear to run almost parallel to one another.

4. The four American "varieties" of *Cyclops viridis* exhibit a constant difference in chromosome number. *C. viridis* (typ. sp.) has twelve, var. *americanus* has ten, var. *parvus* has six, and var. *brevispinosus* has four chromosomes.

5. The six chromosomes of *C. parvus* are in three sizes, there being a pair for each size. The chromosomes of a pair do not necessarily lie together in the spermatogonial or oogonial nucleus.

SPERMATOGENESIS IN *Cyclops americanus*.

6. In the mature Cyclops a Keimpolster distinct from the adult testis may exist.

7. Nuclei in synizesis are smaller, if anything, than the last spermatogonial nuclei. In the testis synizesis is accompanied with only a very slight growth.

8. The nucleus in synizesis resolves itself into five pachytene filaments, from each of which develop two filaments, spirally coiled about one another. The five double filaments uncoil and become the five paired chromosomes of the spermatocyte nucleus.

9. The single elements of the double spermatocyte chromosomes are elongate, dumb-bell-shaped, similar to those of the oocyte.

10. The spermatid chromosomes resolve into a hollow sphere of a reticular chromosome mass. The ripe spermatozoon consists of the spermatid nucleus drawn out into a slightly spiral spindle-shaped body, with fine tapering ends.

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EXPLANATION OF PLATES.

All the figures, except Figs. 23 and 24, were drawn with a Zeiss 1.5 mm. oil-immersion objective and a Zeiss No. 12 compensation ocular. For Fig. 23, ocular No. 8 was used; and for Fig. 24, ocular No. 6. The drawings were made with a Zeiss camera lucida at the level of the base of the microscope, and the reproductions are of the size of the originals.

PLATE I.

Cyclops americanus.

- FIG. 1. Oogonial equatorial plate, showing 10 chromosomes. (Fixation, Flemming; stain, Heidenhain's iron haematoxylin).

Cyclops parvus.

2. Oogonial equatorial plate, showing 6 chromosomes. (Fl.; H.H.)
3. Polar view of oogonial anaphase daughter chromosomes. (Fl.; H.H.)

Cyclops Sp.

4. Nucleus of oviduct egg, late diakinesis showing 5 pairs of chromosomes. (Carnoy; H.H.)

Cyclops americanus.

5. Biserial arrangement of chromosomes in oviduct egg. A partial side view showing only 4 of the 5 paired chromosomes.

Cyclops parvus.

6. Biserial arrangement. Polar view showing 3 paired chromosomes. (Sublimate alcohol (Braun); H.H.)
7. The same, showing smallest chromosome pair abnormally arranged. (Sbl. alc.; Delafield's haematoxylin.)
8. The same. End view of chromosomes.
9. The same. Lateral view of several chromosome pairs to show clear area when present is not always in middle of the chromosome rod. In 9b one rod appears broken in the middle, the other remaining intact. (Sbl. alc.; Delaf. H.)

Cyclops brevispinosus.

10. Biserial arrangement. The egg is lying in the latter end of the oviduct and is compressed from side to side, giving the cytoplasmic area about the chromosomes a spindle shape. There are two pairs of chromosomes. (Carnoy; Delaf. H.)
11. Anaphase I in egg immediately after being laid. (Carnoy; Delaf. H.)

Cyclops parvus.

12. Anaphase I. (Sbl. alc.; H.H.)
13. Anaphase I. (Sbl. alc.; H.H.)

PLATE 2.

Cyclops parvus.

14. First polar body given off. The chromosomes in the egg are turning on their axes preparatory to the next division. (Sbl. alc.; H.H.)
15. Polar view in telophase I. (Picroformol; H.H.)

Cyclops brevispinosus.

16. Polar view in telophase I. (Carnoy; H.H.)

Cyclops parvus.

17. Metaphase II. (Picroformol; H.H.)

Cyclops americanus.

18. Metaphase II. Only 4 of the 5 chromosomes are visible in the section of the egg nucleus. (Carnoy; H.H.)
19. Chromosomes in metaphase II.
20. Telophase II. (Carnoy; safranin.)
21. Second polar body given off. Female pronucleus retreating into egg. (Carnoy; H.H.)

Cyclops parvus.

22. Polar view of contiguous male and female pronuclei preparing for the first segmentation spindle. In one pronucleus the chromosomes are already splitting. (Carnoy; H.H.)

PLATE 3.

SPERMATOGENESIS IN *Cyclops americanus*.
(Fixation, strong Flemming; stain, Heidenhain's
iron haematoxylin).

a. Keimpolster.

- 23. Young *Cyclops* sp.? Keimpolster lying immediately under dorsal wall of cephalothorax.
- 24. Disintegrating Keimpolster at tip of adult testis.

b. Multiplication Zone.

- 25. Resting spermatogonium.
- 26. Spermatogonial prophase, showing 10 chromosomes.
- 27. Spermatogonial metaphase.
- 28. Spermatogonial monaster showing 10 chromosomes.
- 29. Spermatogonial monaster taken from the end of the testis farthest from the Keimpolster.

c. Synizesis and Synapsis Zone.

- 30. Resting spermatogonium.
- 31. Network of spermatogonium forming into filaments and being drawn from nuclear wall.
- 32. Early synizesis figure.
- 33. Later synizesis figure.
- 34. Pachytene stage. Filaments lumpy along their lengths.
- 35. Diplotene stage.

d. Early Diakinesis.

- 36. Five double chromatin filaments. The two filaments to the right extend out of the plane of the section and are therefore only partially shown.
- 37-38. The double chromatin filaments untwisting.

e. Late Diakinesis.

- 39. Two double filaments entirely untwisted. One is much contracted and thickened.
- 40. Definitely formed 5 double chromosomes of the spermatocyte of the first order.
- 41. The same. Abnormal in that the single elements of one of the pairs are separate.

f. Maturation I.

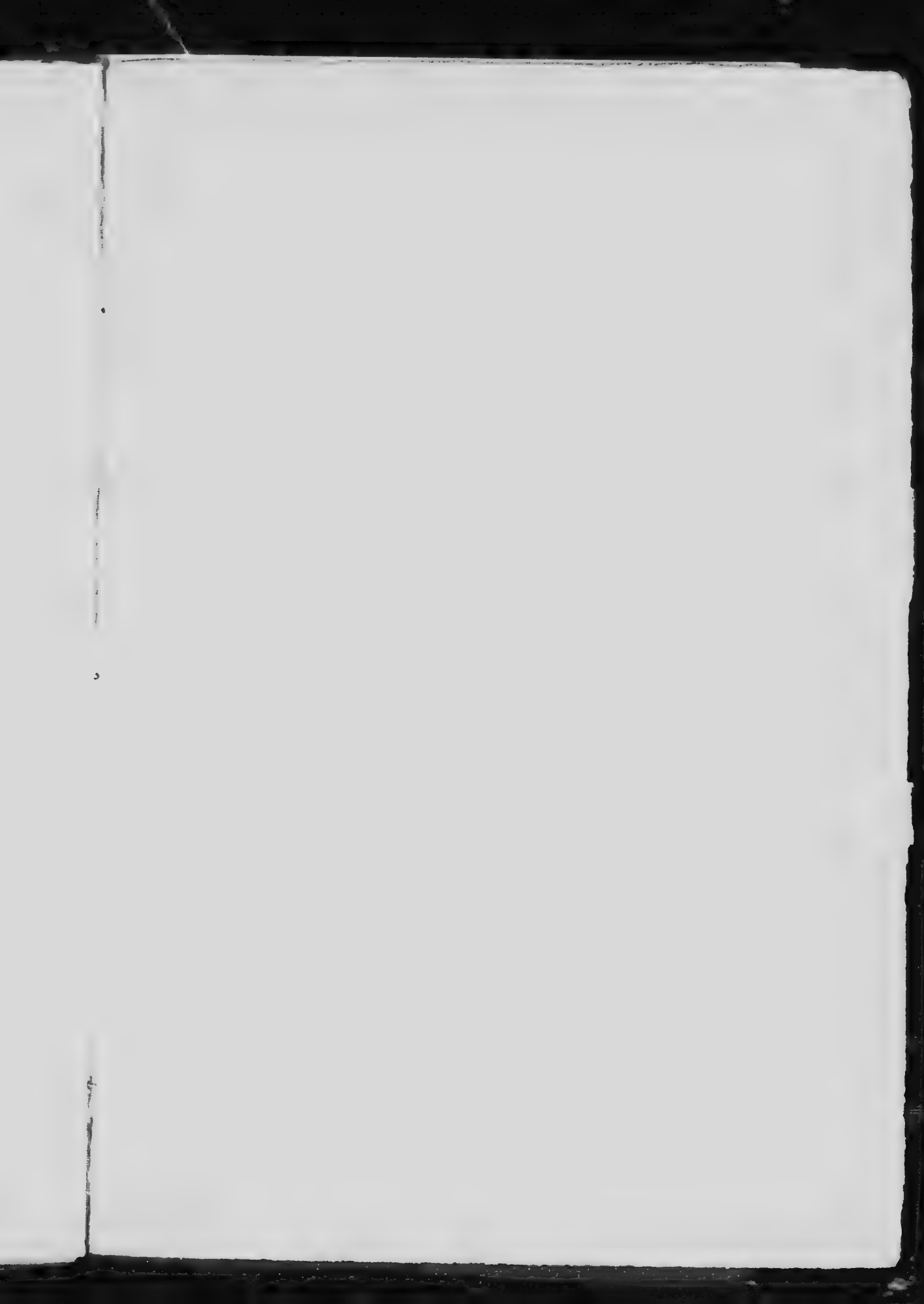
- 42. Anaphase I. Lateral view.
- 43. Chromosomes of metaphase I.
- 44. Late anaphase I. Polar view.
- 45. Telophase I.

g. Maturation II.

- 46. Spermatocyte of 1st = second order.
- 47. Metaphase II.
- 48. Telophase II.

h. Spermiogenesis.

- 49. One spermatid with 5 chromosomes.
- 50. The same. Later stage.
- 51-54. Chromosomes being transformed into a hollow ball of chromatin network which becomes drawn out into a spindle form.
- 55. Mature spermatozoon.
- 55a. Transverse section of a mature spermatozoon.

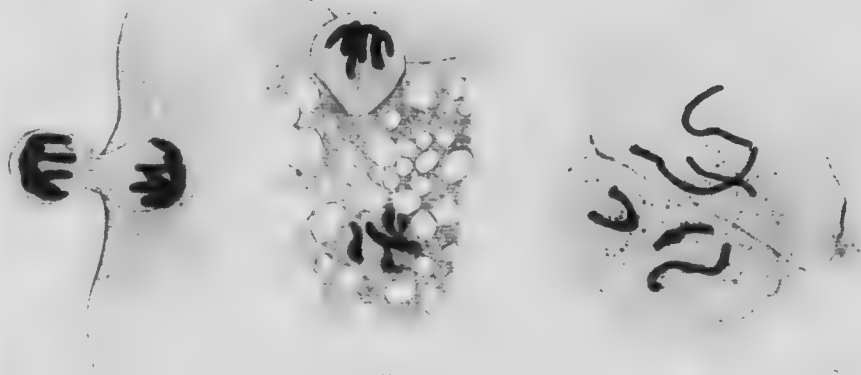


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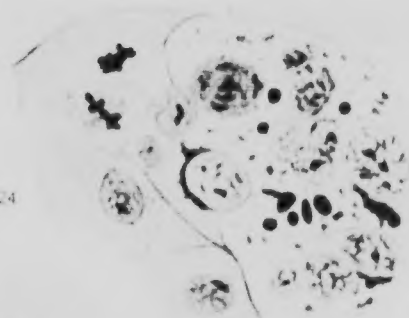
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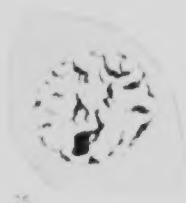




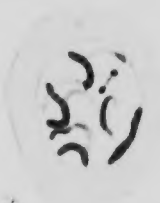
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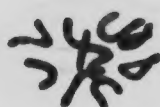
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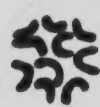
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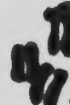
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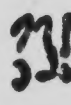
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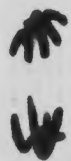
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